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Methamphetamine reduces expression of caveolin-1 in the dorsal striatum: implication for dysregulation of neuronal function

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Abstract

Role of striatal dopamine D1 receptors (D1Rs) in methamphetamine (Meth) taking and seeking is recognized from contingent Meth self-administration studies. For example, Meth increases levels of D1Rs in the dorsal striatum in animal models of Meth addiction, and blockade of striatal D1Rs decreased responding for Meth and reduced Meth priming-induced drug seeking. However, the mechanism underlying enhanced expression of striatal D1Rs in animals self-administering Meth is unknown and is hypothesized to involve maladaptive intracellular signal transduction mechanism via hyperphosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2). D1Rs are predominantly localized to detergent-resistant membrane/lipid raft fractions (MLR fraction), and *in vitro* studies indicate that D1R signaling and recycling is regulated by the MLR-resident protein caveolin-1 (Cav-1), in an endocytotic-dependent manner. Notably, expression of Cav-1 is inversely regulated by ERK1/2 activation, suggesting a signaling interplay among D1Rs, ERK1/2 and Cav-1. We therefore evaluated the effects of extended access Meth self-administration on expression of striatal D1Rs, activated ERK1/2 and Cav-1. We first report that Cav-1 is heavily expressed in neurons located in the dorsal striatum. We also report that extended access Meth produces compulsive-like unregulated intake of the drug, and these behavioral outcomes are associated with enhanced expression of D1Rs, increased activity of ERK1/2, and reduced Cav-1 expression in the dorsal striatum. These data suggest a possible cellular mechanism that involves Cav-1 regulation of D1R expression in response to escalated Meth intake, and how this response of altered D1Rs and enhanced ERK1/2 activation to Meth self-administration contributes to contingent-related processes such as addiction.

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Keywords

Psychostimulant; Animal Models; Neurotoxicity; Dopamine D1 receptor; Caveolin; ERK

Introduction

Dopamine D1 receptors (D1Rs) are most abundant in the striatum (dorsal and ventral striatum) and D1Rs in the dorsal striatum are expressed in the medium-sized spiny striatonigral neurons (MSNs; (Baik, 2013)). Pharmacological studies indicate that Meth (via noncontingent administration) acts as an indirect D1R agonist and produces its rewarding effects through D1Rs in the striatum. For example, D1R antagonism (via SCH23390, a potent D1R antagonist (Millan et al., 2001)) prevented Meth-induced maladaptive intracellular signal transduction mechanism via hyperphosphorylation of ERK1/2, and inhibition of ERK1/2 activity with 2'-amino-3'-methoxyflavone (PD98059; MAPK kinase inhibitor) reduced rewarding properties of Meth (Mizoguchi et al., 2004). These pharmacological studies implicate a strong role of striatal D1Rs in rewarding properties of Meth (Gross et al., 2011), suggesting that alterations in D1Rs could also accelerate the development of Meth-addiction-like behavior (Ares-Santos et al., 2012, Ares-Santos et al., 2013, Toriumi et al., 2014). In support of this hypothesis, contingent Meth via self-administration increases levels of D1R in the dorsal striatum in animal models and human subjects (Worsley et al., 2000, Segal et al., 2005), and blockade of D1Rs in animal models decreased Meth self-administration and reduced Meth priming-induced drug seeking (Bardo et al., 1999, Brennan et al., 2009, Carati and Schenk, 2011). Therefore, it appears that systemic D1R antagonism is neuroprotective, which may correlate with reduced motivation to seek Meth. However, the cellular mechanisms regulating the expression of D1Rs in the dorsal striatum in Meth self-administering animals are unknown. Novel evidence suggests a non-canonical role of membrane lipid raft (MLR) proteins, such as caveolin-1 (Cav-1), in the regulation D1R function (Kong et al., 2007, Voulalas et al., 2011).

In the context of the above hypothesis, Caves are cholesterol-binding and scaffolding proteins within MLRs in neurons, and function as organizers of plasmalemmal signaling molecules including G-protein coupled receptors (assist with receptor internalization and trafficking to the recycling endosomes) (Head et al., 2014, Egawa et al., 2015). Caves are present in three isoforms (Williams and Lisanti, 2004). Cav-1, -2 and -3 are expressed in the central nervous system (Shin et al., 2005), and Cav-1 regulates neuronal signaling (Head et al., 2008, Head et al., 2011). Caves can inhibit signaling via protein-protein interactions (Feron et al., 1998). When up-regulated or overexpressed, Caves promote signaling via enhanced receptor-effector coupling or enhanced receptor affinity (Feron and Balligand, 2006, Head et al., 2011). Cav-1 is involved in multiple cellular processes, including vesicular transport, cholesterol and calcium homeostasis, and signal transduction (Yamamoto et al., 1998). Cav-1 also provides temporal and spatial regulation of signal transduction of D1Rs (Kong et al., 2007, Voulalas et al., 2011). For example, D1Rs are predominantly localized to detergent-resistant membrane fractions (MLR fraction) demonstrating characteristics of proteins associated with Caves (Voulalas et al., 2011). Furthermore, D1R function (signaling and turnover) is regulated by Cav-1 dependent endocytosis (Kong et al., 2007). Cav-1 dependent D1R

internalization is dependent on the integrity of MLRs, because disruption of MLRs (through Cav-1 deficiency) reduces agonist (dopamine)-mediated sequestration of D1R (Kong et al., 2007). However, whether Cav-1 is expressed in the dorsal striatum in neuronal cells is unknown and could assist with defining the role of Cav-1 in regulating Meth-induced dysregulation of D1Rs in the dorsal striatum.

In vitro and *in vivo* studies have begun elucidating the signaling pathways that govern Cav-1 expression. Mechanistic studies conducted *in vitro* in non-neuronal cells demonstrate that Cav-1 gene and protein expression are regulated by ERK1/2 activity, where hyperphosphorylation of ERK1/2 downregulated Cav-1 gene and protein expression by inhibiting Cav-1 promoter activity (Engelman et al., 1999). Notably, the effects of ERK1/2 were abolished by pretreatment with PD98059, suggesting that there is negative regulation between expression of Cav-1 and hyperphosphorylation of ERK1/2 (Engelman et al., 1999). In support of this negative regulation, subsequent *in vivo* study using loss-of-function approaches demonstrated that ERK1/2 is hyperphosphorylated in Cav-1 knockout mice (Cohen et al., 2003), suggesting that Cav-1 can act as an inhibitor of signaling cascades involving ERK (Engelman et al., 1998, Galbiati et al., 1998). Furthermore, Cav-binding motifs are present within the kinase domains of ERKs and several other kinases involved in G-protein coupled signaling (Couet et al., 1997), suggesting a general pattern of negative regulation between Cav-1 and receptor kinases, particularly, ERK1/2 (Okamoto et al., 1998). Therefore, the current study investigated the expression profiles of Cav-1, in the dorsal striatum and examined the alterations in the expression of D1Rs and Cav-1 and activation of ERK1/2 in the dorsal striatum in rats that self-administered Meth via an extended access schedule of reinforcement.

Methods

Animals

Surgical and experimental procedures were carried out in strict adherence to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication number 85–23, revised 1996) and approved by the Institutional Animal Care and Use Committee of The Scripps Research Institute. Thirty eight adult, male Wistar rats (Charles River), weighing 200–250 g at the start of the experiment, were housed two per cage in a temperature-controlled vivarium under a reverse light/dark cycle (lights off 8:00 AM–8:00 PM) and completed the study.

Neurotoxic regimen of Meth (noncontingent Meth)

Rats received systemic injections of Meth [Meth hydrochloride, generously provided by the National Institute on Drug Abuse; (4 mg/kg, s.c.; in saline; n = 4)], four times at 2 h intervals (Gross et al., 2011). The room where the rats were injected was maintained at an ambient room temperature of $23 \pm 1^\circ\text{C}$. Forty-five minutes after the last injection, rats were euthanized by rapid decapitation and brain tissue (right hemisphere) was processed for Western blotting.

Surgery for implanting jugular vein catheters

Nineteen rats underwent surgery for catheter implantation for intravenous self-administration. Rats were anesthetized with 2–3% of isoflurane mixed in oxygen and implanted with a sterilized silastic catheter into the right jugular vein under aseptic conditions. The distal end of the catheter was threaded under the skin to the back of the rat and exited the skin via a metal guide cannula (Galinato et al., 2014). Post surgery care was provided with analgesics (Flunixin) and antibiotics (Cefazolin (Sobieraj et al., 2014)). Catheters were flushed daily with heparinized saline and tested for patency using methohexital sodium (Brevital; (Galinato et al., 2014)).

Training and maintenance on an extended access schedule (contingent Meth)

Four to five days after surgery rats ($n = 19$) were trained to press a lever according to an FR1 schedule of Meth reinforcement (0.05 mg/kg/injection of Meth for every correct response) in operant boxes (Med Associates) under extended access conditions (6h access per day for 17 days). During daily sessions, a response on the active lever resulted in a 4 second infusion (90–100 μ l of Meth), followed by a 20 second time-out period to prevent overdose. Each infusion was paired for 4 seconds with white stimulus light over the active lever (conditioned stimulus [CS]). Response during the time-out or on the inactive lever was recorded but resulted in no programmed consequences. All animals were housed on a reverse cycle (lights off at 8 am) and were transferred from their home cages to their operant chambers between 9 and 10 am. Training on the first and second day was initiated with two-three priming (noncontingent) infusions of Meth during the first ten minutes. Rats were allowed to respond for the remaining fifty minutes without any additional priming. Acquiring Meth self-administration was defined as maintenance of similar number of infusions over 2 days during priming sessions. All animals acquired Meth self-administration and experienced 17 sessions of extended access schedule after the priming sessions. 16–20h after the last session rats were given one infusion of Meth (0.4 mg/kg i.v.) and were euthanized by rapid decapitation 45 min after the injection. The brain tissue for some rats ($n = 10$) was processed for brain meth measures (left hemisphere) or Western blotting (right hemisphere). The remaining animals ($n = 9$) were perfused transcardially (detailed below) and brain tissue was processed for immunohistochemistry.

Quantitative analysis of Meth in the striatum

A separate cohort of rats received one injection of Meth (0.5 mg/kg, 1mg/kg and 2mg/kg, i.p., $n = 2$ each dose) and were euthanized 45 minutes after the injection by rapid decapitation and brain tissue was processed for brain Meth measures (left hemisphere). Control animals were injected with saline (i.p., 1 ml/kg; $n = 5$) and the brain tissue of saline rats was processed for Western blotting (right hemisphere) and brain Meth measures (left hemisphere).

Brain Meth quantification

Rats were injected with Meth (see above; i.v. or i.p.) and were deeply anesthetized (isoflurane) 45 minutes after the injection and killed by decapitation. Brain tissue (enriched in the striatum) was rapidly dissected, and weighed in microcentrifuge tubes. Tissue Meth

concentrations were measured using a previously published liquid chromatography-tandem mass spectrometry assay (Truong et al., 2005, McFadden et al., 2012) with minor modifications by the core facility at The Scripps Research Institute. Briefly, striatal tissue samples were homogenized by sonication in 500 μ l deionized sterile water, and stored at -80°C . The entire sample homogenate was used for analysis.

Preparation of Samples and Standards—On the day of the assay, the homogenates were equilibrated to room temperature. Deuterated Meth (250 ng; METH-d8, Sigma) was added as internal standard to 500 μ l of homogenate. Samples were vortexed for 5s and then made strongly basic by adding alkaline ($\text{pH} > 12$) with 100 μ l of concentrated ammonium hydroxide. Homogenates were extracted into 6 ml of a 4:1 v/v mixture of n-butyl chloride and chloroform for 30 min with gentle shaking. Samples were centrifuged for 10 min at 1200g. The organic phase containing the analyte of interest was transferred to a 16×100 -mm silanized glass screw-capped test tube and evaporated to dryness at 20°C . Residues were reconstituted with 100 μ l of 95:5 formic acid (0.1%) and 5% acetonitrile (v/v) prior to analysis by liquid chromatography/tandem mass spectrometry. A multipoint calibration curve ranging from 1 to 1500 ng/ml homogenate was prepared with drug-free rat plasma and extracted as described above. Analytical intra-assay accuracy and the lack of matrix effect were verified by concurrent analysis of quality control (known standard) samples that were prepared in drug-free (saline injected) rat brain tissue homogenate.

Determination of Meth Concentration—Concentrations of Meth were determined with Agilent 6490 triple quadrupole mass spectrometer with an electrospray ionization source operated in MRM/positive ion mode (Agilent Technologies, Santa Clara, CA). Separation by liquid chromatography was carried out using a 2.1×50 mm Symmetry® LC C18 column (Waters, Milford, MA). Water (with 0.1% formic acid) and acetonitrile (with 0.1% formic acid) were used for the gradient mobile phase; gradient used was as follows – 100/0 at T=0 min, 100/0 at T=1 min, 65/35 at T=5 min, 0/100 at T=6, Off at T=9 min. A 4-min re-equilibration step was included between two consecutive sample injections. Transition states monitored were m/z 150.1 $\rightarrow$ 91.0 for Meth and m/z 158.2 $\rightarrow$ 93.1 for METH-d8. The limit of detection in these experiments was 100 fg/ μ l. Accuracy was within 10% of the Meth concentrations from brain homogenate quality control (known standard) samples.

Western blotting

Procedures optimized for measuring levels of total proteins were employed (Somkuwar et al., 2015). Meth-naïve saline injected rats ($n = 5$), and Meth experienced rats (noncontingent Meth, $n = 4$ and contingent Meth, $n = 10$ rats) were killed via rapid decapitation under light isoflurane anesthesia. Brains were quickly removed, striatum was dissected and flash-frozen till further use. The striatal samples were homogenized on ice by sonication in buffer (320 mM sucrose, 5 mM HEPES, 1 mM EGTA, 1 mM EDTA, 1% SDS, with Protease Inhibitor Cocktail and Phosphatase Inhibitor Cocktails II and III diluted 1:100; Sigma) and heated at 100 degrees C for five minutes. Protein concentration was determined by a detergent-compatible Lowry method (Bio-Rad). Tissue homogenate was stored at -80°C until further use. Samples were mixed (1:1) with a Laemmli sample buffer containing β -mercaptoethanol. Each sample containing protein from one animal was run (20 μ g protein

per lane) on 12% SDS-PAGE gels (Bio-Rad) and transferred to polyvinylidene fluoride membranes (PVDF pore size 0.2 μ m). Blots were blocked with 5% milk (w/v) in TBST (25 mM Tris-HCl (pH 7.4), 150 mM NaCl and 0.1% Tween 20 (v/v)) for 16–20 h at 4 °C and were incubated with the primary antibody D1R (rabbit polyclonal, 1:500, Abcam cat # ab20066, predicted molecular weight 49 kDa, observed band ~48 kDa) for 16–20 h at 4 °C. Blots were washed three times for 5min each with TBST, and then incubated with horseradish peroxidase-conjugated goat antibody to rabbit (1:4,000, BioRad) in TBST for 1 h at room temperature (24 °C). After another three washes for 5 min each with TBST, immunoreactivity was detected using SuperSignal West Dura chemiluminescence detection reagent (Thermo Scientific) and collected using HyBlot CL Autoradiography film (Denville Scientific) and a Kodak film processor. Blots were then probed for β -tubulin (mouse monoclonal, 1:8,000, Santa cruz cat. no. sc-5274, predicted band size 50 kDa, observed band ~50 kDa) to normalize to the quantity of protein loaded in each lane. Densitometry was performed using ImageJ software. X-ray films were digitally scanned at 600 dpi resolution, then bands of interest were selected in identically sized selection boxes within the imaging program. Each band was expressed as a percent of the drug naïve control animals included on the same blot to adjust for blot-to-blot variability.

Perfusions

Meth experienced rats (n = 9, extended access) and Meth and saline naïve controls (n = 4) were fully anesthetized using chloral hydrate (240 mg/kg, i.p.), and transcardially perfused with phosphate-buffered saline (2 min at 15 ml/min) and then with 4% paraformaldehyde (20 min at 15ml/min). The brains were dissected out, postfixed in 4% paraformaldehyde at 4°C for 16–20h, and sliced in 40 μ m sections along the coronal plane on a freezing microtome. Two coronal sections of the rat striatum from each rat (+0.7 to +0.2 mm from bregma; 4 bilateral sections per rat) were mounted on Superfrost® Plus slides and dried overnight and processed for immunohistochemistry.

Antibodies, immunohistochemistry, microscopic analysis, and quantification

The following primary antibodies were used for immunohistochemistry (IHC): Cav-1 (rabbit monoclonal, 1:100, cat# 3267S Cell Signaling Tech), NeuN (mouse monoclonal, 1:50, cat# MAB377 EMD Millipore), GFAP (1:500, chicken polyclonal, cat# ab4674 Abcam), and phosphorylated-p44/42 MAPK (Erk1/2) at Thr202/Tyr204 (mouse monoclonal, 1:100, cat# 9106S Cell Signaling Tech). The sections were pretreated (Mandyam et al., 2004), blocked, and incubated with the primary antibodies followed by cyanine (CY3), FITC, or biotin-tagged secondary antibodies and TSA amplification, and visualized via fluorescence or DAB method. Cav-1/NeuN and Cav-1/GFAP colabeling were assessed by confocal analysis. Confocal analysis of double labeled cells in the dorsal striatum was performed with a Zeiss Axiovert 100 M and LSM510 using a previously published method (Kim and Mandyam, 2014). Briefly, using a 60 $\times$ oil immersion objective, immunoreactive cells were optically sectioned in the z-plane using multitrack scanning (section thickness of 0.5 μ m). Colocalization of antibodies was assessed with the confocal system by analysis of adjacent z-sections (using gallery function and orthogonal function for equal penetration of the antibodies). Cav-1 expression for quantification was determined via densitometry with ImageJ software. Two bilateral sections containing the dorsal striatum (+0.7 to +0.2 mm

from bregma) were used for image capture and Cav-1 density from 4 frames (2 frames per section/2 sections per rat; each frame 0.078 mm²) were analyzed and average density from each animal was used for statistical analysis. Cell counting of pERK1/2 labeled cells was performed visually with a 40x objective (equipped with a 10x eyepiece). Immunoreactive cells from the left and right hemispheres of the dorsal striatum (+0.7 to +0.2 mm from bregma) that were localized in the counting frame (0.078 mm²) were counted and represented as number of cells per mm². All analyses were performed by the observer blind to the study.

Statistical analysis

The Meth self-administration data is expressed as active lever presses and Meth data is also expressed as mean mg/kg Meth self-administered per session. The lever discrimination during Meth self-administration during the 6h session was examined over the 17 escalation sessions using a two-way repeated-measures analysis of variance (ANOVA; session $\times$ lever, with both session and lever as within subject factors) followed by Student-Newman-Keuls *post hoc* test. The pattern of Meth intake is expressed as the mean $\pm$ S.E.M. mg/kg per hour over 6 h sessions and was compared between the first and other sessions. Differences in the rate of responding between the first and other escalation sessions were evaluated using Student-Newman-Keuls *post hoc* test. The Kruskal–Wallis test (non-parametric One-Way ANOVA) was used to determine differences between amount of Meth in the striatum tissue as our sample size ($n = 2$; i.p. groups) did not meet the guidelines for a parametric One-Way ANOVA. Significant ANOVA was followed by Dunn's multiple comparison to determine group differences. Differences in density of proteins or number of immunoreactive cells were analyzed by one-way ANOVA or unpaired *t*-test. Data are expressed as mean $\pm$ SEM and were analyzed using GraphPad Prism. Values of $p \leq 0.05$ were considered statistically significant. Graphs were generated using GraphPad Prism 6.0 software.

Results

Extended access to Meth self-administration resulted in escalation of Meth intake

Rats experienced Meth self-administration for 17 days (Figure 1a–b). Repeated measures two-way ANOVA detected a significant session $\times$ lever interaction (Figure 1a, $F_{16,384} = 7.9$, $p < 0.0001$), a significant effect of lever (Figure 1a, $F_{1,384} = 30.8$, $p < 0.0001$) and a significant effect of session (Figure 1a, $F_{16,384} = 11.38$, $p < 0.0001$). *Post hoc* analysis revealed a significant increase in active lever presses for Meth during the 6h session in extended access animals during session 5–17 compared with session 1. No significant changes were observed in inactive lever responses. Furthermore, Meth intake also increased between session 5–17 compared to session 1 (Figure 1b, $F_{16,237} = 5.08$, $p < 0.001$).

Meth concentrations in the brain increase with increasing dose of the drug; Meth concentrations in the brain do not differ between i.p. and i.v. Meth regimens for a comparable dose

Brain concentrations of Meth were assessed in saline injected animals (controls), Meth injected animals (binge, i.p.) and Meth self-administering animals (0.05 mg/infusion, 6h) challenged with a binge of Meth (binge, i.v.) (Figure 2). Animals that received 2mg/kg i.p.

Meth had about 6ng/mg Meth in the brain tissue, an amount that was higher than that observed in animals injected with 0.5 and 1mg/kg i.p. Meth (Kruskal-Wallis test, $p=0.0025$). Dunn's multiple comparisons test indicated higher levels of brain Meth in 2mg/kg group compared to all other groups ($ps<0.05$) and higher levels of Meth in all groups compared to saline controls ($ps<0.05$). Animals with a history of Meth self-administration showed similar levels of brain Meth levels after a binge (i.v.) compared to drug naïve animals that received binge (i.p.; 0.4mg/kg, i.v. vs. 0.5 mg/kg i.p.).

Neurotoxic regimen of Meth and extended access Meth self-administration enhances D1R expression in the dorsal striatum

To determine whether Meth altered total D1R expression in the dorsal striatum, receptor protein levels were analyzed in drug naïve control, extended access Meth and neurotoxic regimen Meth animals (Figure 3a–b). Data was subjected to one-way ANOVAs to examine the effects of treatment (control vs. Meth groups) and ANOVA showed significant main effect of Meth on D1R expression ($F_{2,18} = 12.53$, $p = 0.001$). Newman-Keuls multiple comparison test demonstrated significant increase in D1Rs in extended access and neurotoxic regimen Meth animals compared with drug naïve controls ($ps < 0.05$; Figure 3c–d). There were no differences between extended access and neurotoxic regimen groups.

Extended access Meth self-administration enhances expression of pERK1/2 in the dorsal striatum

To determine whether Meth altered ERK1/2 activity, expression of pERK1/2 was examined in the dorsal striatum via immunohistochemistry in drug naïve control and extended access Meth animals. Immunostaining of pERK1/2 revealed cells with neuronal morphology with some cells exhibiting dendritic structures in the matrix compartment of the dorsal striatum (Westin et al., 2007). Data was subjected to Unpaired t test to examine the effects of treatment (control vs. Meth). Results indicate a significant increase in the number of pERK1/2 positive cells in the dorsal striatum in extended access Meth animals ($p = 0.024$; Figure 4c).

Caveolin-1 is expressed in neurons in the matrix compartment of the dorsal striatum

In the dorsal striatum, we expected most of the Cav-1 cells to be colabeled with neurons; the colabeling data of Cav-1 and NeuN (a marker for neurons) fit well with expectations based on previous studies performed *in vitro* (Head et al., 2011). Qualitative analysis indicate that nearly all of the Cav-1-labeled cells in the dorsal striatum expressed NeuN and were restricted to the matrix compartment of the striatum (Figure 5a–d), and did not colabel with the astrocytic marker GFAP (Figure 5e–h).

Extended access Meth self-administration reduces Cav-1 expression in the dorsal striatum

To determine whether Meth altered Cav-1 expression in the dorsal striatum, protein expression was analyzed via immunohistochemistry in drug naïve control and extended access Meth animals. Data was subjected to Unpaired t test to examine the effects of treatment (control vs. Meth). Results indicate a significant reduction in Cav-1 levels in extended access Meth animals ($p = 0.053$; Figure 5l).

Discussion

The present experiments demonstrate the compulsive-like unregulated intake of Meth in animals self-administering intravenous Meth via extended access schedule of reinforcement. The findings also report comparable bioavailability of Meth in the brain tissue after systemic or intravenous administration of Meth. The similarities in brain Meth levels also support similar neurotoxicity profiles of Meth with varied routes of administration (chronic compulsive-like Meth self-administration and neurotoxic noncontingent regimen of Meth) which is evident as enhanced D1R expression in the dorsal striatum (Ares-Santos et al., 2013). Concurrent to the enhanced effects on D1R expression, extended access Meth experience enhanced pERK1/2 expression in the dorsal striatum, indicating that excessive compulsive-like self-administration enhanced similar intracellular signal transduction mechanisms as noted in other noncontingent animal models of Meth reward (Mizoguchi et al., 2004, Beaulieu et al., 2006, Bourque et al., 2012, Yan et al., 2014, Zhao et al., 2014). The alterations in D1R expression and pERK1/2 expression were negatively associated with Cav-1 expression, suggesting that Cav-1 may be a unifying regulator of D1Rs and ERK1/2 activity in the dorsal striatum. These findings emphasize the role of non-canonical pathways in regulating D1R receptor trafficking and signaling (Voulalas et al., 2011), which may be hindered after chronic exposure to Meth.

Using noncontingent (neurotoxic regimen) and contingent (extended access Meth self-administration) administration, we attempted to identify alterations in striatal dopamine system markers that uniquely emerge during Meth experience (Shishido et al., 1997, Worsley et al., 2000, Segal et al., 2005, Toriumi et al., 2014). Our results show that the profile of D1R changes in the dorsal striatum with the neurotoxic regimen model was quantitatively similar to that obtained after extended access Meth, supporting prior studies that noncontingent Meth delivery was not a necessary model component to effect D1R changes. This gives credence to the design of self-administration protocol as this experimental paradigm furthers our understanding of the human condition of Meth abuse and addiction (Kitamura et al., 2006). For example, the majority of experimental Meth studies have been conducted using experimenter-delivered drug with the implicit assumption that the resultant Meth concentration in the brain is the critical determinant of behavioral and neurochemical effects. Our findings demonstrate that brain Meth levels did not significantly differ after systemic and intravenous administration of similar doses of Meth. A minor limitation with our brain Meth measures is the use of single binge injection for noncontingent paradigm, whereas brain Meth levels from multiple injection neurotoxic regimen would have strengthened the relationship between neurotoxicity measures and brain Meth levels (Truong et al., 2005). Nevertheless, our findings support the similarities in brain neurochemistry profiles reported above and similar behavioral profiles after Meth experience presented by others after noncontingent and contingent Meth administration (Segal et al., 2005, Hadamitzky et al., 2012). Thus, it appears that while the use of either administration protocol can be justified, contingent approaches, i.e., intravenous self-administration of Meth with extended access to the drug, is increasingly being used with the rationale that the paradigm provides greater face validity with respect to patterns of human Meth abuse (Kitamura et al., 2006, McFadden et al., 2012).

Mechanistic studies indicate that D1R MSNs in the dorsal striatum play a role in promoting both reward and sensitizing responses to psychostimulants (Lobo et al., 2010, Ferguson et al., 2011); however, more comprehensive understanding of D1Rs in mediating the maladaptive behavioral responses in compulsive-like Meth taking and seeking in Meth addicted animals is unknown (Ahmed and Koob, 2004, Segal et al., 2005). For example, studies from noncontingent Meth administration indicate that Meth increases D1R expression and D1Rs in the dorsal striatum are indicated to play a role in Meth-induced hyperphosphorylation of ERK1/2 and Meth reward (Shishido et al., 1997, Bardo et al., 1999, Mizoguchi et al., 2004, Toriumi et al., 2014). Notably, studies from contingent Meth administration also demonstrate increases levels of D1R in the dorsal striatum in preclinical models and human subjects (Worsley et al., 2000, Segal et al., 2005), and systemic blockade of D1Rs decreased responding for Meth self-administration and reduced Meth priming-induced drug seeking (Bardo et al., 1999, Brennan et al., 2009, Carati and Schenk, 2011). An increase in D1R levels and perhaps function may explain why extended access Meth does not increase dopamine response to Meth in the dorsal striatum, and this regulation by Meth may also support preferential effect of D1-like antagonists on the maintenance of Meth self-administration (Stefanski et al., 1999, Brennan et al., 2009, Le Cozannet et al., 2013). Therefore, it appears that systemic D1R antagonism is neuroprotective which may correlate with reduced motivation to seek Meth (Lacan et al., 2013, Le Cozannet et al., 2013, Parsegian and See, 2014).

Increase in D1R expression in the dorsal striatum was associated with increased activity of ERK1/2. Activity of ERK1/2 has been observed in acute responses to psychostimulants (Valjent et al., 2000), and in the development of long-term changes of gene expression, synaptic plasticity, and locomotor responses following repeated exposure to amphetamines (Beaulieu et al., 2006, Bourque et al., 2012, Yan et al., 2014, Zhao et al., 2014). Although a limitation of the current study is the lack of mechanistic relationship between D1R and ERK1/2 activity, and ERK1/2 activity and development of addiction-like behavior, some of this relationship has been previously explored in the dorsal striatum with noncontingent administration of Meth (Mizoguchi et al., 2004). In addition, the same study demonstrated that the mechanistic relationship between D1Rs and ERK1/2 activity in the dorsal striatum directed behavioral responses to Meth. Furthermore, another study has demonstrated direct evidence for ERK1/2 activity in regulating behavioral responses to amphetamines, indicating that intracellular mechanisms regulating ERK1/2 activity can influence biological actions of psychostimulants (Beaulieu et al., 2006). Such evidence suggests that activation of ERK1/2 by Meth could assist with long lasting neuroadaptations by Meth which could be related to maladaptive behavioral responses to Meth in an extended access paradigm.

Recent reports from experiments conducted in adult rats demonstrate that D1Rs in the striatum and cortical areas are localized almost exclusively to detergent-resistant membrane fractions (Voulalas et al., 2011), where MLRs and Cavs are expressed (Mandyam et al., 2015). Most notable, is the alteration in the expression patterns of D1Rs to detergent-soluble membrane fractions devoid of Cavs after noncontingent cocaine administration, suggesting enhanced expression/proportion of D1Rs after psychostimulant insult (Voulalas et al., 2011). The altered subcellular distribution of D1Rs post cocaine treatment is hypothesized to initiate and secure maladaptive plasticity and promote functional alterations in D1R signal

transduction. In support of this hypothesis, studies from *in vitro* experiments demonstrate that D1Rs are localized in caveolae, and disruption of caveolae resulted in enhanced signaling of D1Rs (Kong et al., 2007). The findings from our study extend these reports and demonstrate that Meth disrupts Cav-1 expression in the dorsal striatum, and these effects were associated with enhanced expression of D1Rs. Furthermore, reduced Cav-1 is associated with enhanced activity of ERK1/2 in the same subjects, demonstrating a relationship between the two proteins. While it is conceivable that these events may be causally linked (Engelman et al., 1998, Engelman et al., 1999), such experiments in the context of Meth addiction-like behavior are an interesting future pursuit.

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Highlights

- Extended access to Meth produces unregulated Meth intake
- Chronic Meth enhances expression of dopamine D1 receptors in the striatum
- Enhanced D1 receptor expression is associated with hyperactivity of ERK1/2
- Hyperactivity of ERK1/2 is associated with reduced expression of Caveolin-1

Figure 1.

Operant self-administration behavior indicated as lever responses (a) and total amount of Meth consumed (b). Data are expressed as mean $\pm$ S.E.M.. * $p < 0.05$ vs. active lever responses in (a), # $p < 0.05$ vs. day 1 of self-administration in (a, b).

Figure 2.

Amount of Meth measured in the brain after i.p. or i.v. Meth injections. # $p < 0.05$ vs. saline, * $p < 0.05$ vs. 2mg/kg dose. Data are expressed as mean $\pm$ S.E.M..

Figure 3.

Schematic representation of Meth self-administration performed over 17 sessions (a) and binge Meth treatment (b). Red arrow indicated termination of experiment by rapid decapitation and brain tissue collection. Representative immunoblots of D1R in control (drug naïve, C), extended access Meth (E), and binge Meth (B) animals. β -tubulin is indicated as a loading control. Quantitative analysis of D1R density from controls, extended access Meth and binge Meth animals, indicated as percent change from control. * $p < 0.05$ vs. control. Data are expressed as mean $\pm$ S.E.M..

Figure 4.

Qualitative (a–b) and quantitative analyses of ERK1/2 activity via pERK1/2 immunohistochemistry. pERK1/2 staining was rarely seen in control (a) animals and was seen distributed in the matrix compartment of the striatum of Meth (a) animals. Arrowhead points to darkly stained immunoreactive cells. Scale bar in (a) is 10 μ m applies a–b. (c) Quantitative analysis of the total number of pERK1/2 positive cells per mm² of the dorsal striatum. * $p < 0.05$ vs. controls. Data are expressed as mean $\pm$ S.E.M..

Figure 5.

Confocal double labeling of Cav-1 with NeuN (a–d) and GFAP (e–h). Cav-1 is indicated in red (CY3) and NeuN and GFAP are indicated in green (FITC). Orthogonal z section (d, h) indicates equal penetration of all antibodies shown in x–z and y–z planes. Arrowheads in (a–h) point to Cav-1 immunoreactive cells. Arrowhead in (d) points to a double labeled Cav-1-NeuN cell, and in (h) points to a Cav-1 cell that does not express GFAP. Scale bar in (a) is 30 μ m applies a–d, is 40 μ m applies e–h. (i) Cartoon representation of striatal section (0.7 mm from bregma) used for immunohistochemical analysis and a square box indicates the area used for the photomicrographs indicated in (j–k). (j–k) DAB immunoreactivity of Cav-1 expression in the dorsal striatum from control (j) and Meth (k) animal. Scale bar in (k) is 80 μ m applies j–k. (l) Densitometric analysis of Cav-1 immunoreactivity in the dorsal striatum. * $p < 0.05$ vs. controls. Data are expressed as mean $\pm$ S.E.M..